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Cannabinoids, Electrophysiology, and Retrograde Messengers: Challenges for the Next 5 Years

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ABSTRACT

Most of the behavioral effects of cannabis and its active ingredients, the cannabinoids (Δ^9THC being the most abundant of these), appear to be mediated by cannabinoid receptors. Endogenous cannabinoids (endocannabinoids) are lipid mediators that activate these same cannabinoid receptors. Elegant work from several laboratories over the past 5 years has established that endocannabinoids, possibly acting as retrograde messengers, mediate several forms of neuronal plasticity. Endocannabinoid-mediated neuronal plasticity is common, apparently occurring at all neurons that express cannabinoid receptors. Thus, it is likely that Δ^9THC produces its effects by interacting with endocannabinoid-mediated neuronal plasticity, though whether it does so cooperatively or antagonistically remains an open question. In this review we will briefly discuss the work establishing endocannabinoids as mediators of neuronal plasticity and then present evidence that a major effect of Δ^9THC may be to antagonize the actions of endocannabinoids.

KEYWORDS: neuronal plasticity, endocannabinoid, long-term depression, calcium, cannabis

INTRODUCTION

A fundamental objective in cannabinoid research is to understand how cannabis and its primary psychoactive component, Δ^9THC, produce their characteristic psychoactive effects. Rapid advances in the cannabinoid field over the past 5 years have put us tantalizingly close to this goal. Here we will review those advances as they relate to synaptic physiology. Modern neuroscience asserts that consciousness and alterations in consciousness are likely mediated by neuronal activity and modulation of this activity. As cannabis and Δ^9THC produce prominent alterations in mood, emotion, memory, and perception, it is likely these substances are producing their effects by influencing neuronal activity. Below, we will present evidence from several recent studies showing that both endogenous and exogenous cannabinoids have profound effects on neuronal activity, particularly synaptic transmission. We will then argue that it is the interactions between endogenous and exogenous cannabinoids that underlie the psychoactivity of cannabis and its constituents.

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THE ENDOCANNABINOID SYSTEM

The endocannabinoid system is a ubiquitous neuromodulatory system with wide-ranging actions whose extent and mechanisms are still being elucidated. It comprises cannabinoid receptors, endogenous cannabinoids (endocannabinoids, eCB's), and enzymes responsible for their production, transport, and degradation. Of the several cannabinoid receptors identified either pharmacologically or molecularly, this review will focus on CB1 receptors, as they are the most abundant and best characterized. CB1 receptors are G-protein-coupled receptors (GPCRs), preferentially activating $G_{i/o}$ proteins.[1] Their activation leads to inhibition of adenylyl cyclase, inhibition of certain voltage-sensitive calcium channels (predominately, those found presynaptically), activation of inwardly-rectifying potassium channels, and activation of mitogen-activated protein (MAP) kinase.[1] CB1 receptors are abundantly expressed in the nervous system, particularly in "higher" brain regions, including the cortex, amygdala, and hippocampus.[2] Here the highest levels of CB1 cannabinoid receptors are found on the terminals of cholecystokinin (CCK) positive GABAergic interneurons.[3,4] Given their location and the signaling pathways they activate, it is predicted that CB1 receptors will suppress neurotransmission and neuronal excitability. Indeed, several studies have shown this to be the case.[5-7]

Given the locations and actions of cannabinoid receptors, one would expect the presence of an endogenous ligand, a prediction that has been borne out. Specifically, 2 families of endocannabinoids have been extensively characterized. The first are amides of arachidonic acid (or closely related polyunsaturated fatty acids) and ethanolamide. The amide class of endocannabinoids is typified by arachidonoyl

ethanolamide, also known as anandamide.[8] The second family is arachidonic acid esterified to the 2 position of glycerol (2-arachidonoyl glycerol, or 2-AG).[9] The synthetic pathways for both of these endocannabinoids are quite complex, and the interested reader is encouraged to consult one of several excellent reviews on the topic.[1,2] The key feature of the synthesis of these endocannabinoids, for the purposes of this review, is that they exist as preformed precursors in the membrane and thus are enzymatically produced, or "made on demand" in response to specific signals, such as increases in intracellular calcium or activation of phospholipase C β by $G_{q/11}$ metabotropic receptors. How (and even if) endocannabinoids move from the extracellular space to the interior of a cell for degradation remains a subject of controversy.[10,11] Nonetheless, evidence from several studies suggests that there is an inhibitable process that facilitates bidirectional transport of endocannabinoids across the cell membrane.[12,13] Degradation of the 2 classes of endogenous cannabinoids in vivo also occurs by distinct pathways: fatty acid amide hydrolase primarily degrades anandamide and its congeners, while monoacyl glycerol lipase degrades 2-AG and related esters.[14,15] There is also evidence for oxygenase metabolites of endocannabinoids as will be discussed in a later section. While these are the 2 best-characterized families of endocannabinoids, ongoing studies suggest that arachidonic acid conjugated to several molecules, such as amino acids, forms bioactive compounds, many of which have activity at cannabinoid receptors.[16] The exact physiological role and metabolic pathways for the synthesis and degradation of these compounds remain to be delineated.

Cannabinoids Inhibit Neurotransmission

There is ample evidence for the proposition that cannabinoids inhibit neurotransmitter release and neurotransmission. Sheldon Roth's group published one of the earliest studies, predating even the pharmacological characterization of cannabinoid receptors.[17] They found that Δ^9THC and related compounds stereospecifically suppressed electrically evoked ilieal contractions. Subsequent work from several labs has extended these studies to central nervous system (CNS) neurons and provides a mechanistic basis for attenuation of neurotransmission by CB1 activation.[5] Presumed mechanisms generally invoke inhibition of presynaptic (eg, N- and P/Q-type) calcium channels[18,19] but may also involve activation of potassium channels and CB1-mediated direct inhibition of the vesicular release machinery.[6]

Cannabinoids and Neural Plasticity

Five years ago a fundamental question in the cannabinoid field was how to relate activity-dependent synthesis of endocannabinoids with their demonstrated ability to inhibit neurotransmission. While studying the phenomenon of depolarization-induced suppression of inhibition (DSI), Rachel Wilson, then in Roger Nicoll's lab, worked out an answer to this question, thereby offering a remarkable insight into cannabinoid signaling.[20] In DSI, strong depolarization of a postsynaptic neuron produces a signal that diffuses to the presynaptic terminal and transiently attenuates gamma-aminobutyric acid (GABA) release from that terminal. A similar phenomenon has been reported for glutamate release in excitatory neurons and is designated DSE (depolarization induced suppression of excitation). Through a careful series of experiments, Rachel Wilson's work identified cannabinoid receptors as being necessary for DSI and suggested that endocannabinoids served as the retrograde messenger. The proposed scheme was that after their production in the postsynaptic cell, endocannabinoids diffused across the synaptic cleft to activate presynaptic CB1 receptors, thereby inhibiting neurotransmitter release. Contemporaneously with this report, studies from both the Regehr and Kano labs revealed that a similar phenomenon involving endocannabinoids and cannabinoid receptors occurred at excitatory synapses in the cerebellum and hippocampus.[21,22] Subsequent work from a large number of groups has shown that DSI and DSE are widespread phenomena, occurring at most synapses expressing CB1 receptors.[23]

How does depolarization bring about the production of endocannabinoids? Depolarization will open voltage-dependent calcium channels, increasing calcium inside the neuron. Early studies by Daniele Piomelli and his colleagues found that anandamide production was enhanced as intracellular calcium increased.[24] Similarly, 2-AG synthesis is increased by manipulations that increase intracellular calcium.[9] However, more recent studies suggest that release of calcium from intracellular stores during depolarization is necessary for DSI or DSE to occur and that entry of extracellular calcium plays a minor role, at least at certain synapses.[25,26]

DSE and DSI are quite transitory, suggesting a very active termination process. Endocannabinoids are rapidly hydrolyzed, making degradation a likely mechanism to terminate DSE and DSI. Insight into which endocannabinoids mediate DSI and DSE can be gained by investigating if inhibition of anandamide or 2-AG degradation alters the time course of DSE or DSI. Inhibition of fatty acid amide hydrolase (FAAH) generally does not prolong DSE or DSI[27]; however, inhibition of either monoacylglycerol (MAG) lipase or cyclooxygenase does,[27,28] suggesting a role for these latter 2 enzymes in the degradation of the endocannabinoids involved in DSE and DSI. Of interest, where it has been examined, endocannabinoid uptake does not seem to be involved in terminating DSE or DSI.[26,29,30] Indeed, it is inhibition of the putative endocannabinoid membrane transporter that appears to inhibit endocannabinoid-mediated plasticity, suggesting that the transporter might be involved in the egress of endocannabinoids from the postsynaptic cell.

The above discussion has reviewed the "classical" features of DSI and DSE. However, there are several additional aspects of these processes that should be kept in mind. The first is that in the electrophysiological recordings that are used to study DSI and DSE, what is being measured is the release of small neurotransmitters that activate ligand-gated ion channels. Yet in the hippocampus, GABA is co-localized with CCK in CB1-expressing terminals. Thus, activation of CB1 receptors would be expected to decrease the release of both GABA and CCK. Indeed, this seems to be the case.[31] Therefore, models that attempt to explain the effects of DSI and similar phenomena must also take into account the implications of inhibiting the release of neuromodulators such as CCK. For example, CCK acting through its receptors would be expected to enhance neuronal activity. Thus, the balance between increased excitability secondary to decreased GABA release on the one hand and reduced excitability secondary to decreased CCK release on the other will determine the net effect of DSI on neuronal excitability. The second major point is that neurons are not the only cell type in the CNS that make endocannabinoids—glial cells can produce large quantities of 2-AG, anandamide, and related compounds.[32] The relative contribution of neuronally and glially produced endocannabinoids in the CNS remains to be examined. Finally, the above discussion has been framed in the context of presynaptic CB1 receptors. Despite ambiguous anatomical evidence, there is strong functional evidence for somatic CB1 receptors. Activation of these receptors, possibly by opening inwardly rectifying potassium channels, suppresses neuronal firing. Thus a relatively localized release of endocannabinoids can have an effect over a considerably greater distance. Examples of a network effect of locally released endocannabinoids have been found in both the cerebellum and cerebral cortex.[33,34]

In addition to a short-term neuronal plasticity mediated by endocannabinoids released following depolarization, a similar inhibition can be induced by activation of certain $G_{q/11}$-linked receptors. This process has been designated metabotropic-induced suppression of excitation (or inhibition) and is abbreviated MSE (or MSI). The receptors most closely linked to MSE and MSI are group I metabotropic receptors (often mGluR5) and muscarinic receptors (M1 and M3). For MSE and MSI, the following proposed sequence of events would lead to 2-AG production: activation of the GPCR stimulates a phospholipase C β, producing IP_3 and diacylglycerol (DAG). The fatty acid at the one position in DAG is then cleaved by a diacylglycerol lipase, producing 2-AG. MSE/MSI are different from DSE/DSI in that the former do not seem to require a rise in intracellular calcium (however, intracellular calcium may increase secondary to IP3 released by phospholipase C during endocannabinoid synthesis).[35,36] The depolarization- and metabotropic-induced processes occur via independent pathways, and thus when both are activated they can be synergistic in their inhibition of neurotransmission. For example, depolarizations or agonist concentrations, which on their own would not produce a detectable endocannabinoid release, may in combination produce substantial endocannabinoid synthesis and inhibition of neurotransmission.[37] Therefore, it has been proposed that activation of phospholipase C and production of endocannabinoids serves as a coincidence detector between neuronal depolarization and activation of metabotropic receptors linked to $G_{q/11}$.[37]

Endocannabinoids also participate in at least one form of long-term synaptic plasticity, a type of long-term depression (eLTD). eLTD is evident at certain synapses following prolonged low frequency stimulation of excitatory synaptic inputs.[30,38,39] Here the process appears to involve the following events. Sustained release of glutamate strongly stimulates postsynaptic mGluR5 receptors. This causes synthesis of endocannabinoids that activate presynaptic CB1 receptors, which sets in motion as-yet-unidentified signaling machinery, which ultimately leads to a sustained decrease in the efficiency of neurotransmitter release, a characteristic of eLTD. Several features of eLTD should be noted. The first is that while activation of presynaptic CB1 receptors is necessary for eLTD induction, it is not necessary for the maintenance of eLTD.[38] Thus, CB1 receptors in eLTD are activating different signaling pathways than those activated in DSI. In DSI/DSE, the prominent effect appears to be inhibition of presynaptic calcium channels, a process that requires the continued presence of endocannabinoids. The second is that the stimulated excitatory pathway is not necessarily the one that shows eLTD. Rather it may be an adjacent pathway. For example, in the hippocampus, prolonged low frequency stimulation of the excitatory Schaffer collaterals leads to LTD of the *inhibitory* pathway onto the same dendritic shafts.[40] In contrast, in the nucleus accumbens, the glutamatergic fibers projecting to this nucleus from the prefrontal cortex contain cannabinoid receptors and their low frequency stimulation leads to LTD of this excitatory synaptic connection to the medium spiny neurons of the accumbens.[41]

Interactions Between Δ^9THC and Endocannabinoid Signaling

The above experimental evidence coupled with numerous other studies strongly implicates endogenous cannabinoids as mediators of specific forms of short- and long-term synaptic plasticity. An interesting and significant question is: "How would smoked cannabis modify endocannabinoid signaling?" Cannabis is a complex mix of chemicals; however, the dominant psychoactive component is Δ^9THC. One expectation that could be inferred from the above studies is that Δ^9THC might simply substitute for endogenous cannabinoids and activate the same signaling pathways. Despite the attractive simplicity of the above hypothesis, the actual situation is likely to be more complex.

From several studies, 2-AG appears to be the major endocannabinoid mediating both the transient and persistent forms of endocannabinoid-mediated neuronal plasticity.[26,28,42] However, Δ^9THC (and anandamide) has a much lower intrinsic efficacy than does 2-AG and will show partial agonism depending on receptor and/or effector density.[43] Thus, depending on CB1 density, 2-AG concentration, the particular repertoire of G proteins activated, and the intracellular signaling pathway, Δ^9THC might well antagonize endogenous 2-AG action. This is perhaps a radical theory, but it is supported by several lines of evidence, both in vitro and in vivo. From in vitro experiments we know that Δ^9THC antagonizes both synthetic and endocannabinoid-mediated inhibition of synaptic transmission as well as DSE and MSE in cultured autaptic neurons[26,44] (Straiker and Mackie, unpublished observations, 2005). The in vivo evidence is that high doses of the CB1 antagonist only modestly attenuate the subjective effects of cannabis intoxication.[45,46] This stands in contrast to the situation with opiates, where very low doses of naloxone will readily block the euphoric effects of opiates such as morphine, methadone, or heroin. Thus, Δ^9THC may be producing its effects not by indiscriminately mimicking endogenous cannabinoids but by antagonizing at least some of their actions.

Of interest, the interactions between Δ^9THC and endogenous cannabinoids become more complex when examining the effect of chronic Δ^9THC. Overnight treatment of cultured neurons with Δ^9THC or short-term administration of Δ^9THC in animals leads to tolerance to the effects of cannabinoids, both on neurotransmission (in cultures or in slices prepared from chronically treated mice) and behavior (in animal models). Thus, even though the acute effects of endocannabinoids are blocked by Δ^9THC, chronic treatment with this compound leads to the development of tolerance indistinguishable from that produced by chronic treatment with highly efficacious cannabinoids. Therefore, it is likely that Δ^9THC, despite its low intrinsic efficacy and inability to inhibit neurotransmission in cultured neurons, is nonetheless capable of activating the CB1 receptor signaling pathways that lead to desensitization. Potential mechanisms for such a Δ^9THC-induced desensitization include CB1 receptor internalization, CB1 receptor sequestration, CB1 receptor phosphorylation, or some combination of all 3.[47-51]

SUMMARY

Studies over the last 5 years have given us a tremendously increased understanding of the basic mechanisms of endocannabinoid modulation of neuronal signaling. It is clear that endocannabinoids are made in response to neuronal activity and are centrally involved in some forms of both long and short forms of neuronal plasticity. Furthermore, the interactions between Δ^9THC and endocannabinoids are more complex than initially suspected. The challenge for the next 5 years will be to identify which actions of endocannabinoids occur in what parts of the brain and to integrate these roles into a broader understanding of the CNS. As emerging results from investigating the interactions between Δ^9THC and endocannabinoids illustrate, many of these will not be what we expect.

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REFERENCES

1. Howlett AC, Barth F, Bonner TI, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev*. 2002;54:161-202.

2. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev*. 2003;83:1017-1066.

3. Katona I, Sperlagh B, Sik A, et al. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci*. 1999;19:4544-4558.

4. Tsou K, Mackie K, Sanudo-Pena MC, Walker JM. Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience*. 1999;93:969-975.

5. Hajos N, Katona I, Naiem SS, et al. Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci*. 2000;12:3239-3249.

6. Hoffman AF, Lupica CR. Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J Neurosci*. 2000;20:2470-2479.

7. Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci*. 1996;16:4322-4334.

8. Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*. 1992;258:1946-1949.

9. Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature*. 1997;388:773-778.

10. McFarland MJ, Barker EL. Anandamide transport. *Pharmacol Ther*. 2004;104:117-135.

11. Hillard CJ, Jarrahian A. Cellular accumulation of anandamide: consensus and controversy. *Br J Pharmacol*. 2003;140:802-808.

12. Fegley D, Kathuria S, Mercier R, et al. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc Natl Acad Sci USA*. 2004;101:8756-8761.

13. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science*. 1997;277:1094-1097.

14. Cravatt BF, Lichtman AH. Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. *Curr Opin Chem Biol*. 2003;7:469-475.

15. Dinh TP, Freund TF, Piomelli D. A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids*. 2002;121:149-158.

16. Bradshaw HB, Walker JM. The expanding field of cannabimimetic and related lipid mediators. *Br J Pharmacol*. 2005;144:459-465.

17. Roth SH. Stereospecific presynaptic inhibitory effect of delta9-tetrahydrocannabinol on cholinergic transmission in the myenteric plexus of the guinea pig. *Can J Physiol Pharmacol*. 1978;56:968-975.

18. Wilson RI, Kunos G, Nicoll RA. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron*. 2001;31:453-462.

19. Twitchell W, Brown S, Mackie K. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol*. 1997;78:43-50.

20. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*. 2001;410:588-592.

21. Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron*. 2001;29:717-727.

22. Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron*. 2001;29:729-738.

23. Diana MA, Marty A. Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). *Br J Pharmacol*. 2004;142:9-19.

24. Di Marzo V, Fontana A, Cadas H, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature*. 1994;372:686-691.

25. Isokawa M, Alger BE. Retrograde endocannabinoid regulation of GABAergic inhibition in the rat dentate gyrus granule cell. *J Physiol*. 2005;567:1001-1010.

26. Straiker A, Mackie K. Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. *J Physiol*. 2005;569:501-517.

27. Kim J, Alger BE. Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nat Neurosci*. 2004;7:697-698.

28. Storozhuk MV, Ivanova SY, Piomelli D. Presence of depolarization-induced suppression of inhibition in a fraction of gabaergic synaptic connections in rat neocortical cultures. *Zh Vyssh Nerv Deiat Im I P Pavlova*. 2005;55:581-585.

29. Fortin DA, Trettel J, Levine ES. Brief trains of action potentials enhance pyramidal neuron excitability via endocannabinoid-mediated suppression of inhibition. *J Neurophysiol*. 2004;92:2105-2112.

30. Gerdeman GL, Ronesi J, Lovinger DM. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci*. 2002;5:446-451.

31. Beinfeld MC, Connolly K. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci Lett*. 2001;301:69-71.

32. Walter L, Franklin A, Witting A, et al. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J Neurosci*. 2003;23:1398-1405.

33. Bacci A, Huguenard JR, Prince DA. Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature*. 2004;431:312-316.

34. Kreitzer AC, Carter AG, Regehr WG. Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. *Neuron*. 2002;34:787-796.

35. Kim J, Isokawa M, Ledent C, Alger BE. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J Neurosci*. 2002;22:10182-10191.

36. Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron*. 2001;31:463-475.

37. Hashimotodani Y, Ohno-Shosaku T, Tsubokawa H, et al. Phospholipase Cbeta serves as a coincidence detector through its Ca2+ dependency for triggering retrograde endocannabinoid signal. *Neuron*. 2005;45:257-268.

38. Robbe D, Alonso G, Manzoni OJ. Exogenous and endogenous cannabinoids control synaptic transmission in mice nucleus accumbens. *Ann N Y Acad Sci*. 2003;1003:212-225.

39. Chevaleyre V, Castillo PE. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron*. 2003;38:461-472.

40. Chevaleyre V, Castillo PE. Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron*. 2004;43:871-881.

41. Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci USA*. 2002;99:8384-8388.

42. Melis M, Perra S, Muntoni AL, et al. Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J Neurosci*. 2004;24:10707-10715.

43. Luk T, Jin W, Zvonok A, et al. Identification of a potent and highly efficacious, yet slowly desensitizing CB1 cannabinoid receptor agonist. *Br J Pharmacol*. 2004;142:495-500.

44. Kelley BG, Thayer SA. Delta 9-tetrahydrocannabinol antagonizes endocannabinoid modulation of synaptic transmission between hippocampal neurons in culture. *Neuropharmacology*. 2004;46:709-715.

45. Huestis MA, Gorelick DA, Heishman SJ, et al. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry*. 2001;58:322-328.

46. Huestis MA, Preston KL, Heishman SJ. Antagonism of inhaled cannabis by single and multiple doses of SR141716 (rimonabant) in male subjects with a history of cannabis use. 2004 Symposium on the Cannabinoids; June 22-27, 2004; Icettam-Paestum, Italy. Burlington, VT: ICRS; 2004: 86.

47. Coutts AA, Anavi-Goffer S, Ross RA, et al. Agonist-induced internalization and trafficking of cannabinoid CB1 receptors in hippocampal neurons. *J Neurosci*. 2001;21:2425-2433.

48. Hsieh C, Brown S, Derleth C, Mackie K. Internalization and recycling of the CB1 cannabinoid receptor. *J Neurochem*. 1999;73:493-501.

49. Jin W, Brown S, Roche JP, et al. Distinct domains of the CB1 cannabinoid receptor mediate desensitization and internalization. *J Neurosci*. 1999;19:3773-3780.

50. Kouznetsova M, Kelley B, Shen M, Thayer SA. Desensitization of cannabinoid-mediated presynaptic inhibition of neurotransmission between rat hippocampal neurons in culture. *Mol Pharmacol*. 2002;61:477-485.

51. Sim LJ, Hampson RE, Deadwyler SA, Childers SR. Effects of chronic treatment with delta9-tetrahydrocannabinol on cannabinoid-stimulated [35S]GTPgammaS autoradiography in rat brain. *J Neurosci*. 1996;16:8057-8066.